The 3rd AsiaBlight International Meeting

Pathogen effectors as probes for improving late blight resistance in potato

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Outlines

1. Plant disease problems
2. Plant disease resistance
3. Oomycete RXLR effectors
4. Use of *P. infestans* RXLR effectors
5. Effector targets as novel source of disease resistance
   - CAD7: conserved plant target of multiple Avr3a effectors
   - StMKK1：a potato MAPK cascade protein target of effector E49
   - PPIase FKBP15-2: novel component of ER-mediated immunity
6. Conclusions

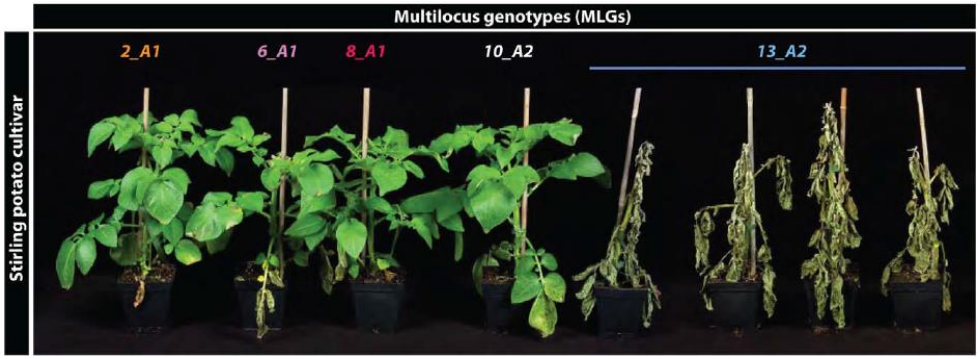
1. Plant disease problems

- Potato late blight: *Phytophthora infestans*

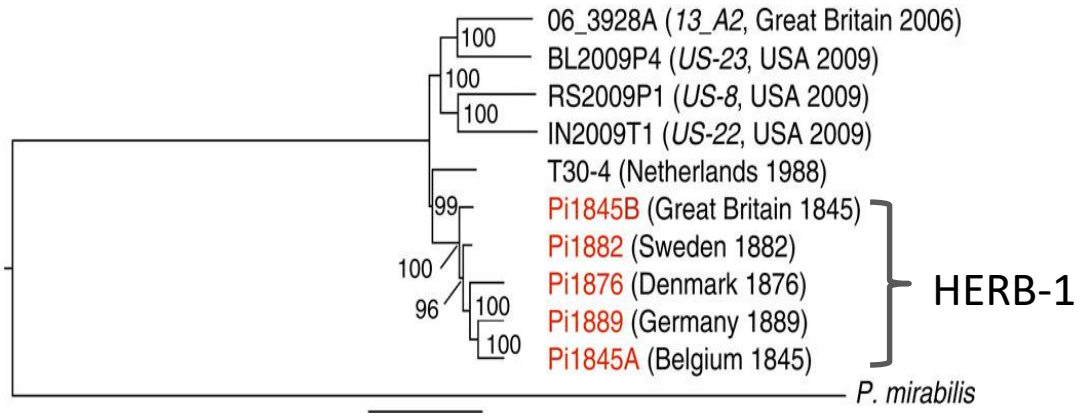
- The Irish Famine in 1840s
- Loss of genotype-specific disease resistance
- Disease control relies on fungicide application

1. Plant disease problems

➢ *Phytophthora infestans*: the *R* gene destroyer

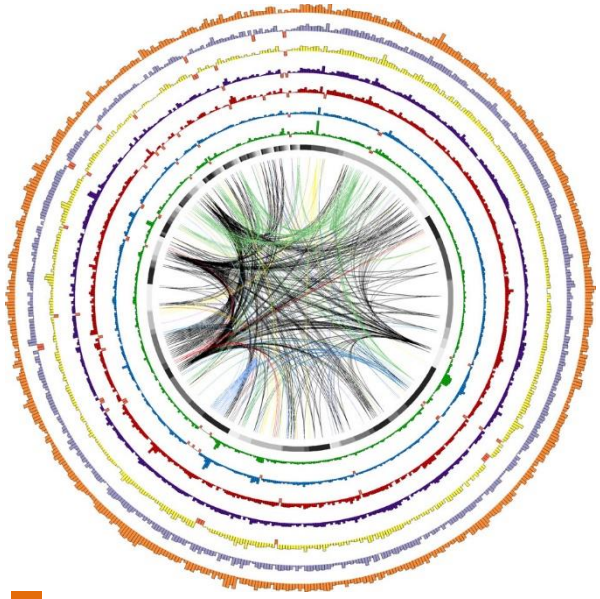


Cooke et al. 2012. PLoS Pathogens



Martin et al. 2013. Nature Communications

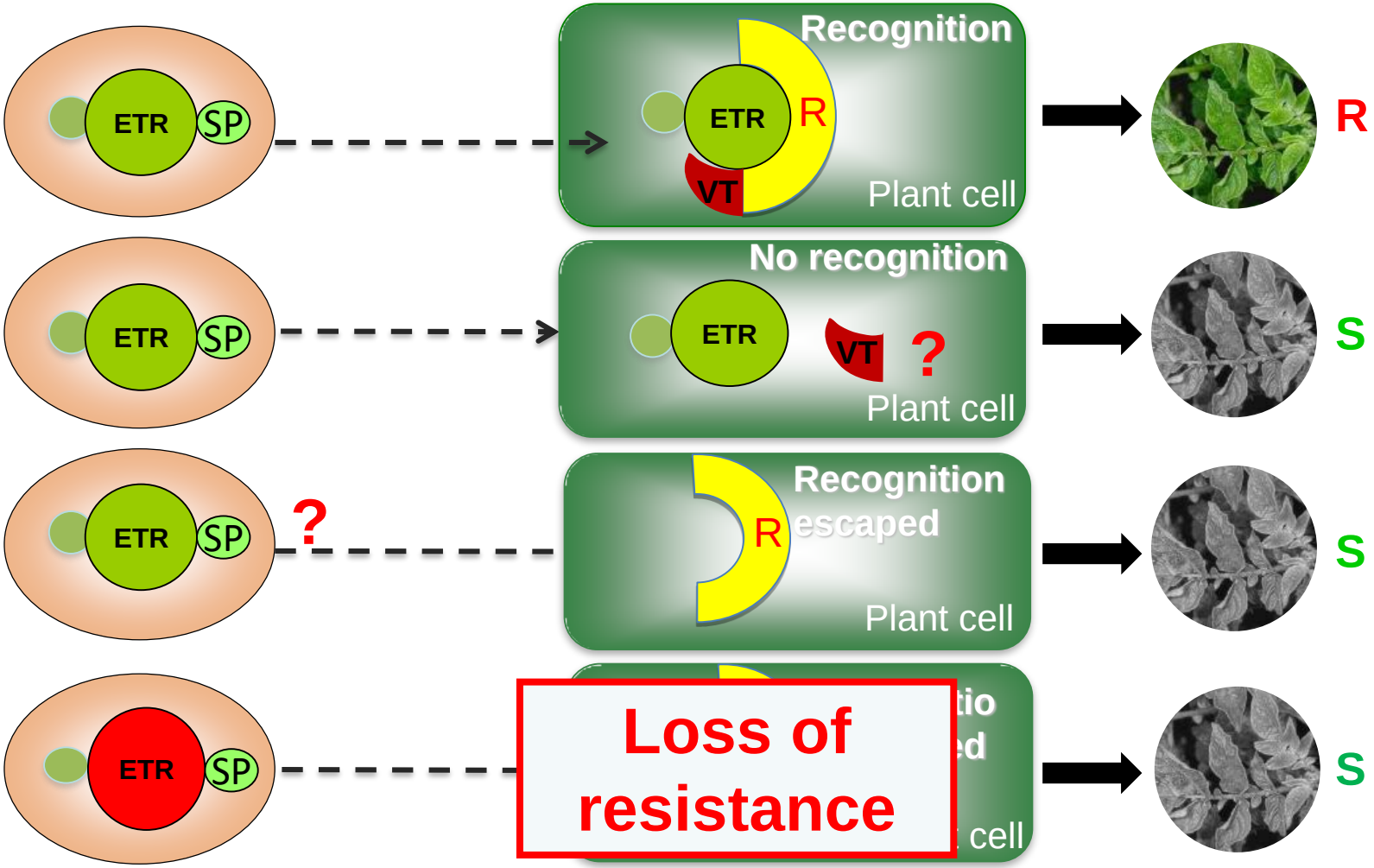
RXLR effectors



T30-4
06_3928A (*13_A2*)
RS2009P1 (*US-8*)
BL2009P4 (*US-23*)
IN2009T1 (*US-22*)
Pi1889 (HERB-1)
Pi1845A (HERB-1)

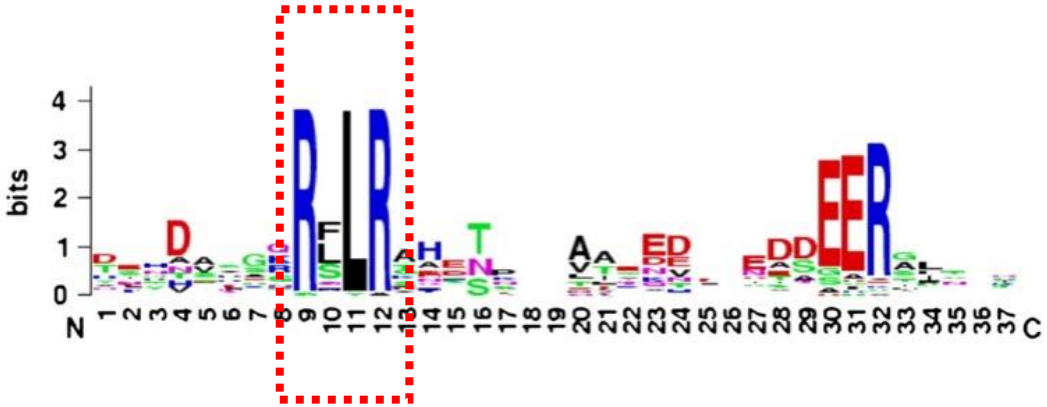
2. Plant disease resistance

- Disease resistance is achieved by plant recognition of pathogen effectors



3. Oomycete RXLR effectors

➢ Oomycete RXLR effectors



Avr1b

Shan et al. 2004. MPMI （Avr1b）

Allen et al. 2004. Nature （ATR13）

Armstrong et al. 2005. PNAS （Avr3a）

Rehmany et al. 2005. Plant Cell （ATR1）

Pathogen/Plant *Avr* Gene	Protein
Hyaloperonospora arabidopsidis/Arabidopsis thaliana	
Atr1	RXLR
Atr5	RXLR-like
Atr13	RXLR
Atr39	RXLR
Phytophthora infestans/Solanum species	
Avr1	RXLR
Avr2	RXLR
Avr3a	RXLR
Avr4	RXLR
AvrBlb2	RXLR
IPI-O	RXLR
AvrVnt1	
Phytophthora sojae/Glycine max	
Avr1a	RXLR
Avr1b-1	RXLR
Avr1c	RXLR
Avr1d	RXLR
Avr1k	RXLR
Avr3a/5	RXLR
Avr3b	RXLR
Avr3c	RXLR
Avr4/6	RXLR

Anderson et al. 2015.MPMI 28:1063-1072

3. Oomycete RXLR effectors

➢ Oomycete RXLR effectors: numerous and divergent

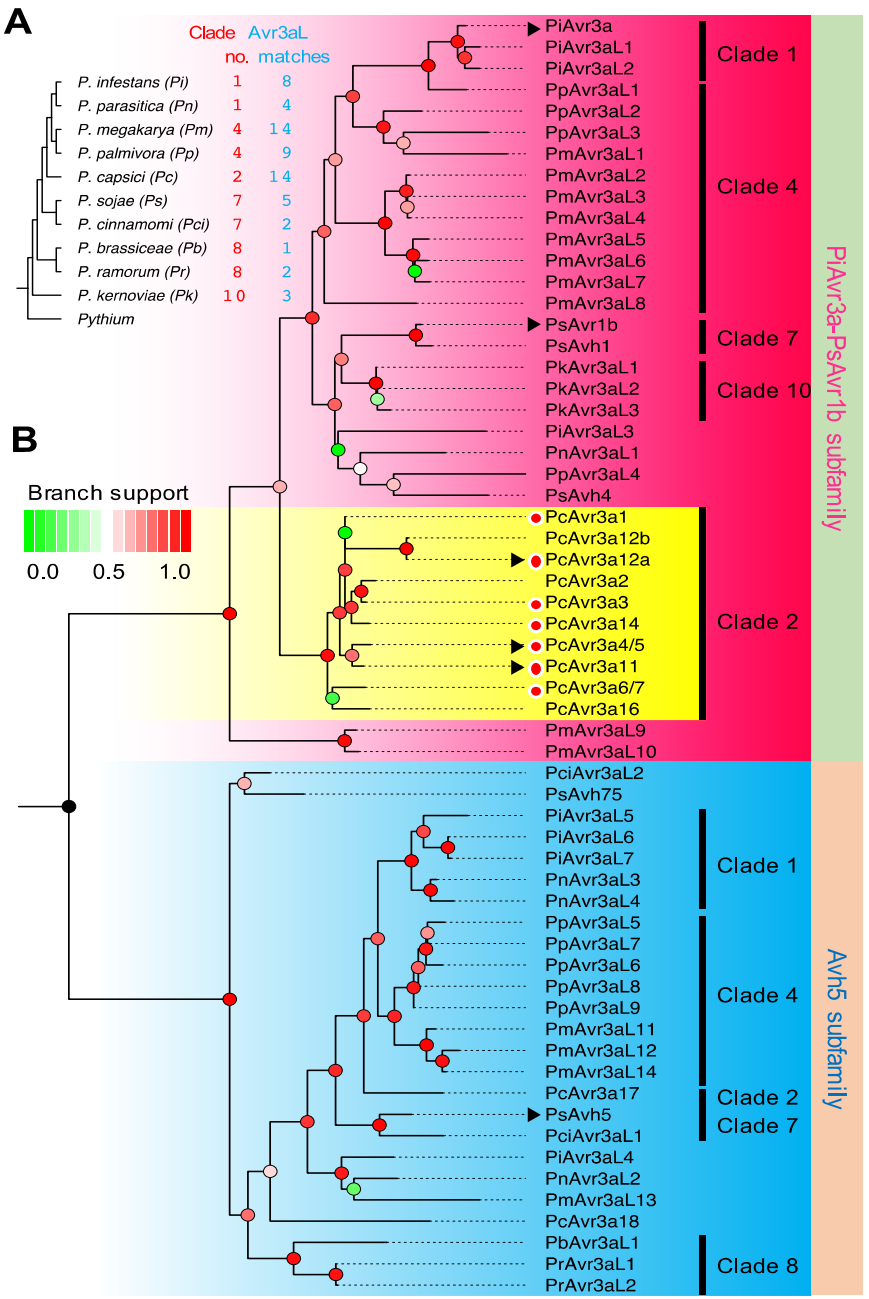
	Phytophthora sojae	*Phytophthora ramorum*	*Phytophthora capsici*	*Phytophthora infestans*	*Hyaloperonospora parasitica*	*Magnaporthe grisea*	*Ustilago maydis*
Genome size (Mb)	**95**	**65**	**65**	**240**	**75**	**40.3**	**20.5**
Gene number	**19,027**	**15,743**	**12,011**	**22,658**	**15,743**	**12,841**	**6,902**
Secreted protein genes	**1,464**	**1,188**	**ND**	**ND**	**ND**	**739**	**426**
RXLR effector genes	**402**	**531**	**420**	**563**	**147**	**--**	**--**

From: Tyler et al. 2006. Science 313: 1261-1266.
Haas et al. 2009. Nature 461: 393-398.
Dean et al. 2005. Nature 434: 980-986.
Kamper et al. 2006. Nature 444: 97-101.
Baxter et al. 2010. Science 330: 1549-1551.
Anderson et al. 2015.MPMI 28:1063-1072

3. Oomycete RXLR effectors

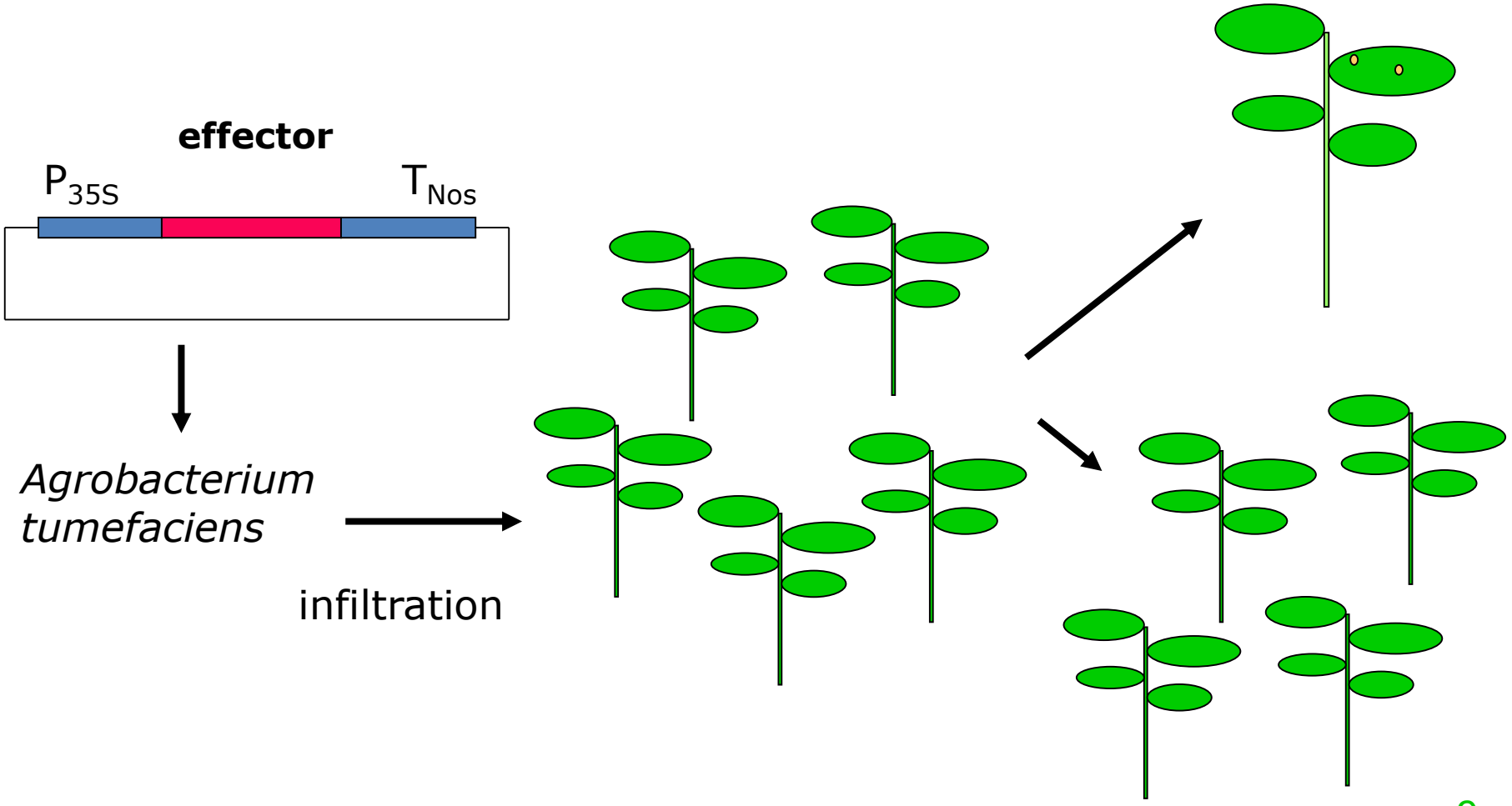
- Avr3a-like effectors are distributed widely across *Phytophthora* species

The expanded *Avr3a* gene family in the genome of *P. capsici*



4. Use of *P. infestans* RXLR effectors

➢ Identification of resistance genes：Agro-infiltration



4. Use of *P. infestans* RXLR effectors

➢ *R* gene evaluation for specificities

- ➢ Recognition of an effector by multiple R proteins of different origin；
  - ➢ *P. infestans* effector PiAvr2 can be recognized by R2 from *Solanum demissum*, RpiBlb3 and RpiAbpt from *S. bulbocastanum*, and the RpiSnk1 from *S. schenckii*;
  - ➢ *P. infestans* effector PiAvrBlb1 can be recognized by RpiBlb1 of *S. bulbocastanum*, RpiSto1 of *S. stoloniferum*, and RpiPta1 of *S. papita*
- ➢ Reduced genetic materials for disease resistance breeding.

➢ *R* gene evaluation for durability

- ➢ Population genetic variations of effectors：the presence of multiple variants of an effector suggests less durability of cognate R gene；
- ➢ The durable *RpiBlb1* and *RpiBlb2* from *S. bulbocastanum* are capable of recognizing multiple variants of cognate effectors.

4. Use of *P. infestans* RXLR effectors

➢ Genetic dissection of *R* genes in a variety or promising line

R genes in potato variety Sarpo Mira

- showed promising field performance against late blight;
- effector recognition;
- carries at least 5 *R* genes.

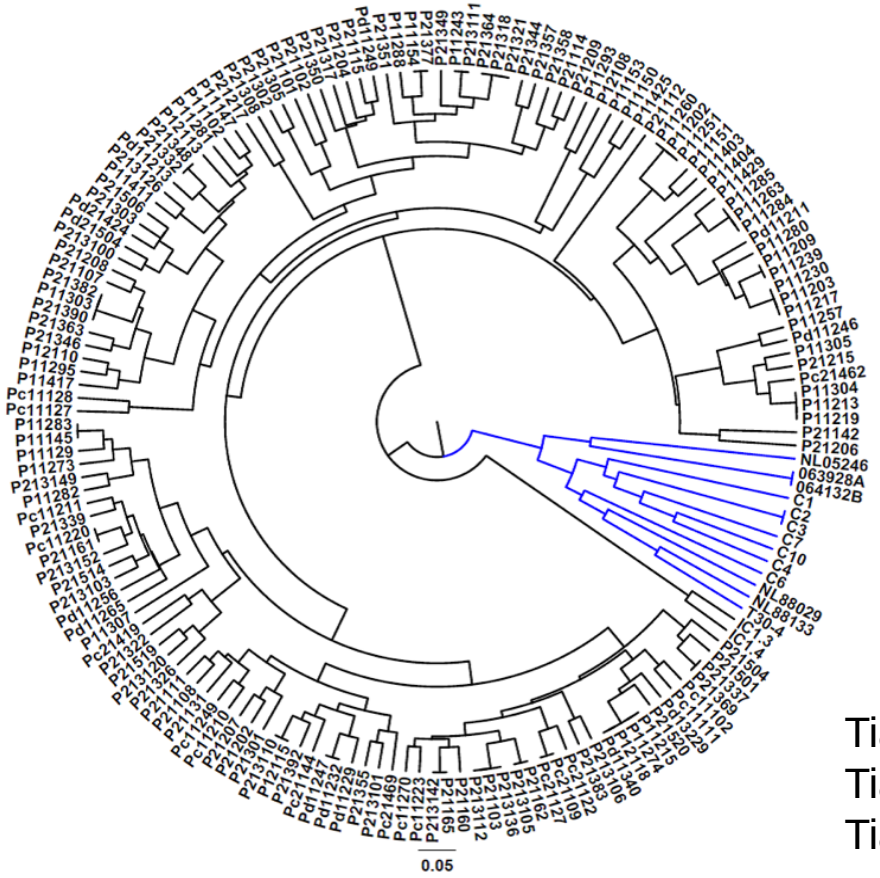
Rietman et al. 2012. MPMI

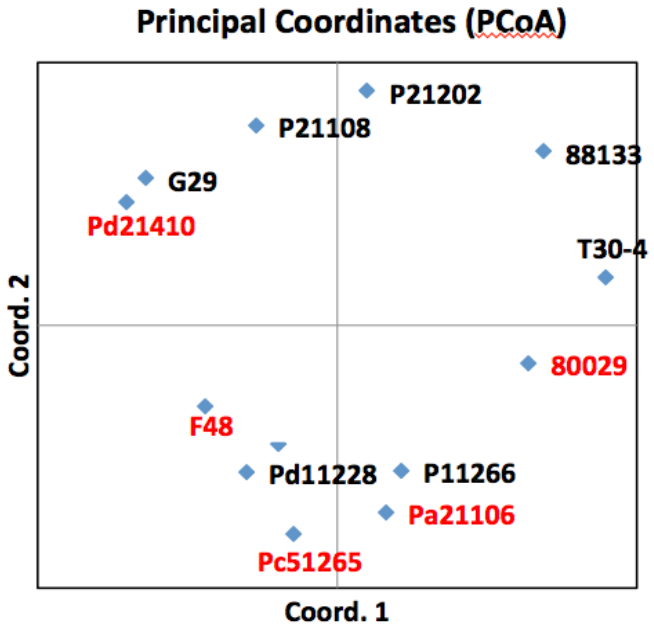
4. Use of *P. infestans* RXLR effectors

- Identification and creation of durable *R* genes against late blight
  - Identification and use of core *P. infestans* effector gene: screening germplasms for new *R* genes；
  - Use of all but limited virulent variants of an effector to screen germplasms for new *R* genes;
  - Genetic engineering for new *R* genes capable of recognizing all but limited virulent effector variants.

4. Use of *P. infestans* RXLR effectors

- Core effectors of *P. infestans*: towards durable resistance

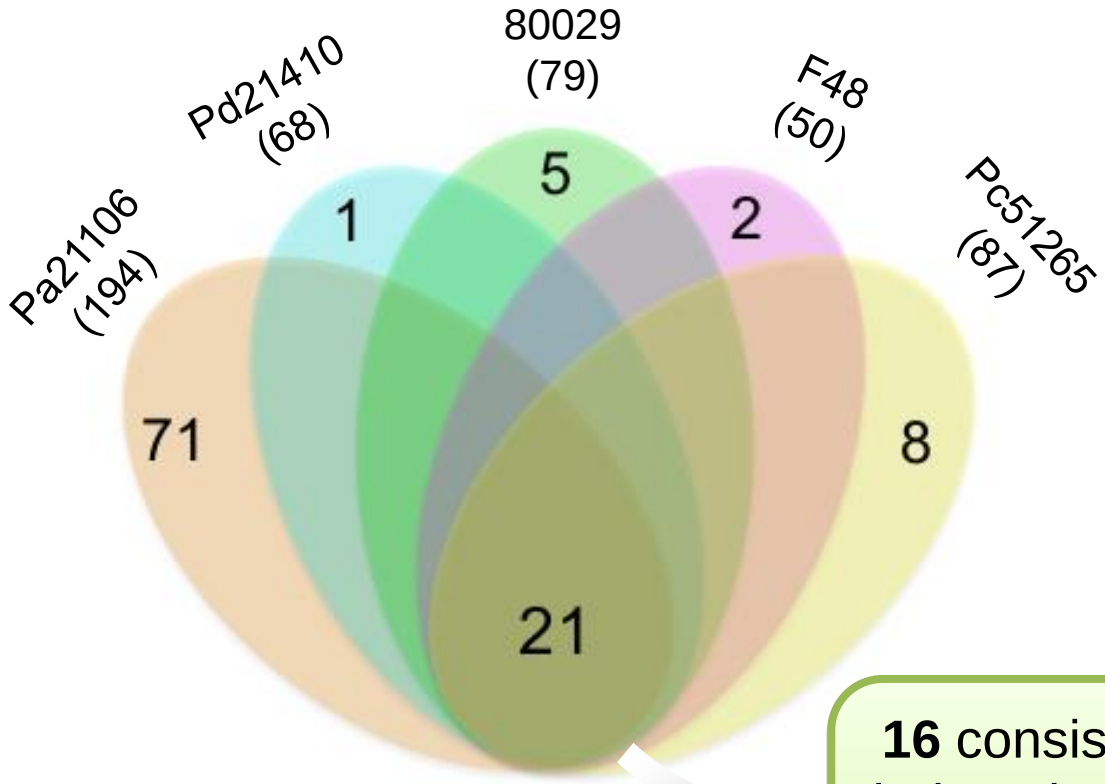


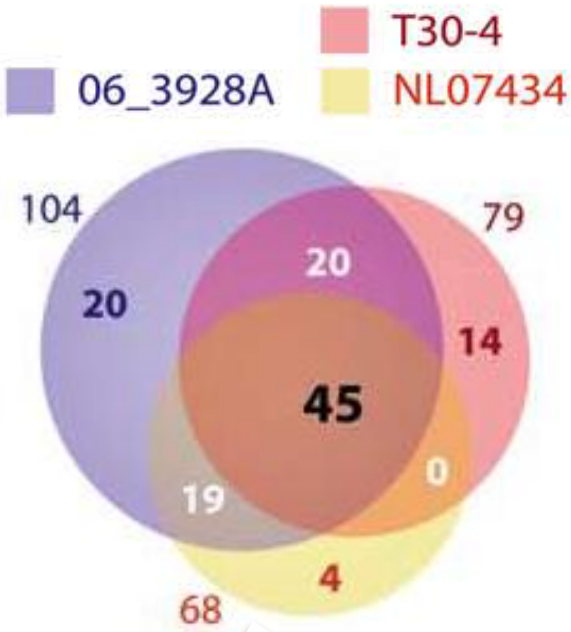


Tian et al. 2015 .Plant Pathology 64: 200-206.
Tian et al. 2015. Phytopathology 105: 771-777.
Tian et al. 2016. Plant Pathology 65:17-25

4. Use of *P. infestans* RXLR effectors

➢ Core effectors of *P. infestans*: towards durable resistance



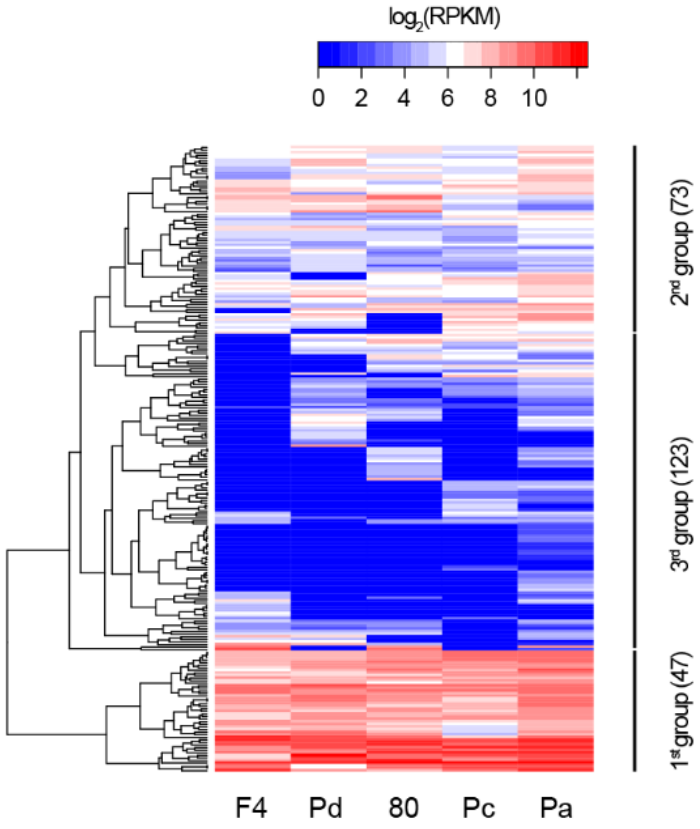


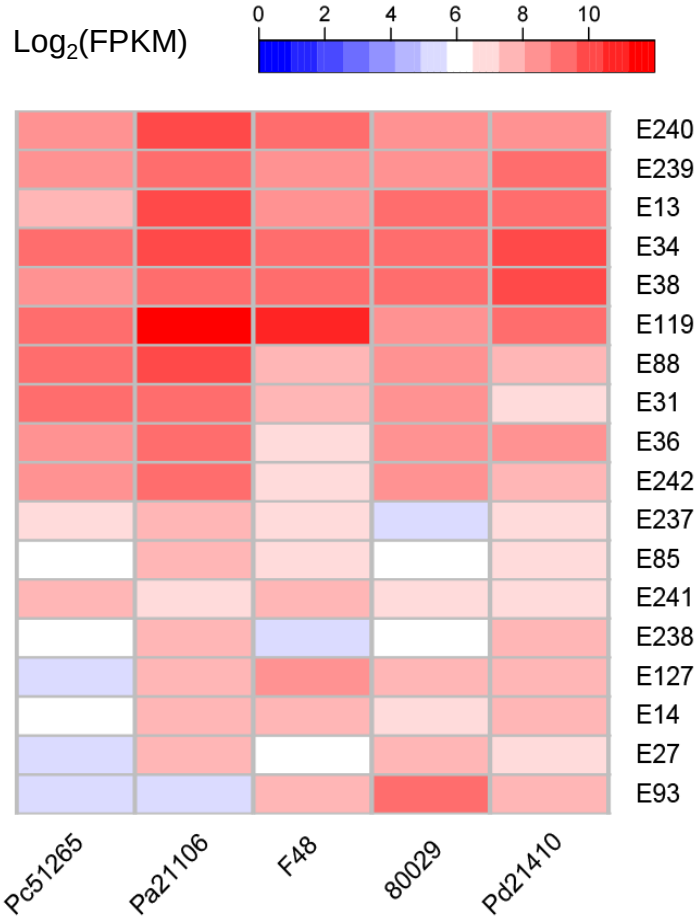
16 consistently induced RXLRs in at least 6 strains

Cooke et al. 2012
Yin et al. 2017

4. Use of *P. infestans* RXLR effectors

- Core effectors of *P. infestans*: towards durable resistance

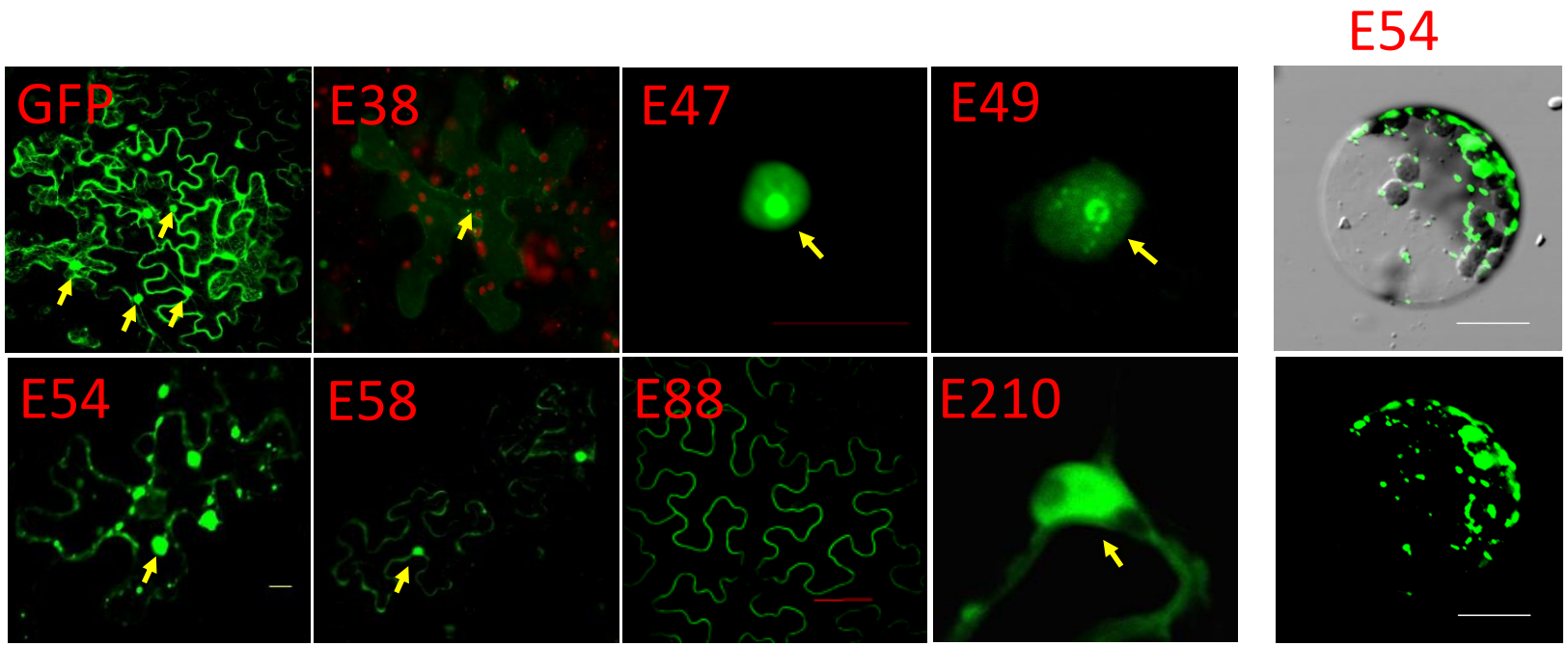




Yin et al. 2017. Front. Plant Sci.

4. Use of *P. infestans* RXLR effectors

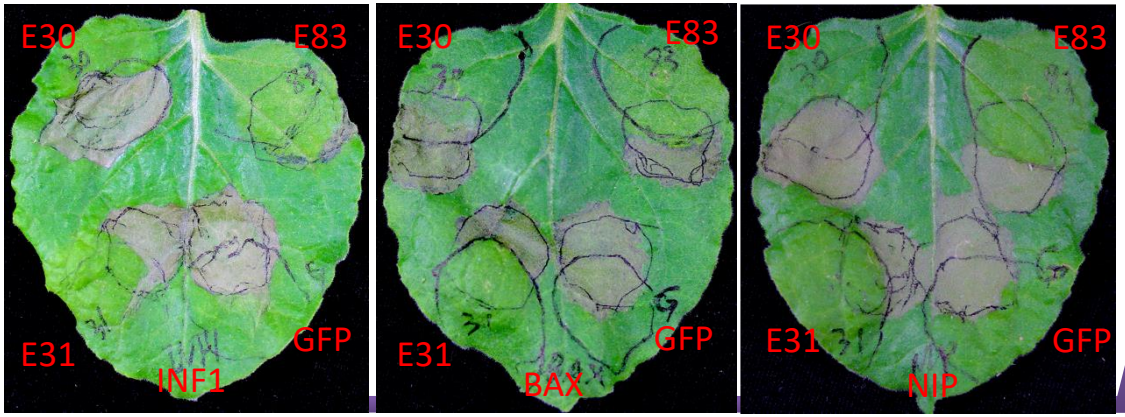
- Core effectors of *P. infestans*: towards durable resistance



Huang et al. unpublished

4. Use of *P. infestans* RXLR effectors

➢ Core effectors of *P. infestans*: virulence factors



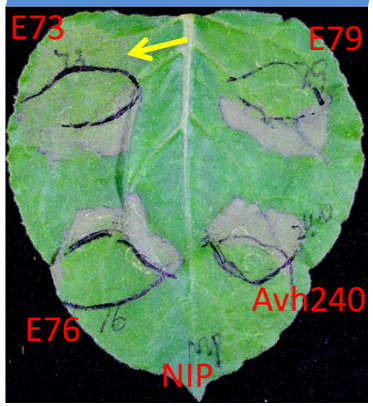
BAX: Apoptosis regulator

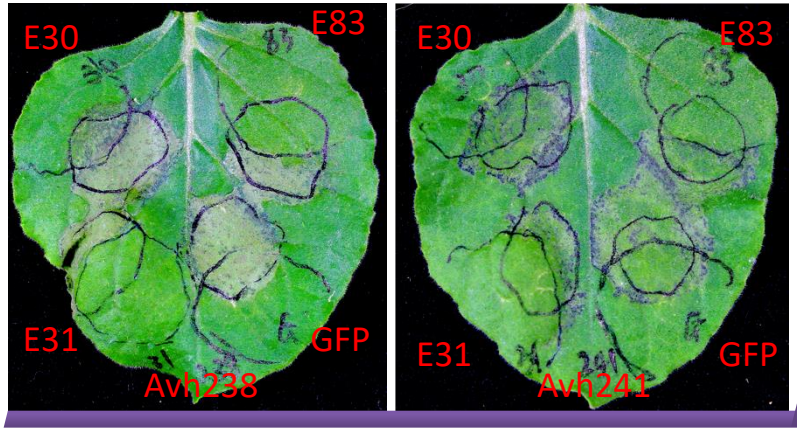
NIP: *P. sojae* PAMP

INF1: *P. infestans* PAMP

PTI suppression

Induction of cell death





Avh238: *P. sojae* effector

Avh241: *P. sojae* effector

ETI suppression

4. Use of *P. infestans* RXLR effectors

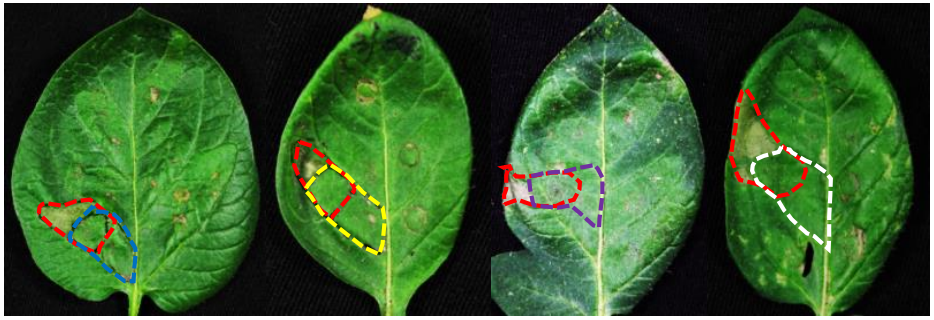
- Candidate core effectors: promotes plant susceptibility

A

GFP E27 E31 E34 E38

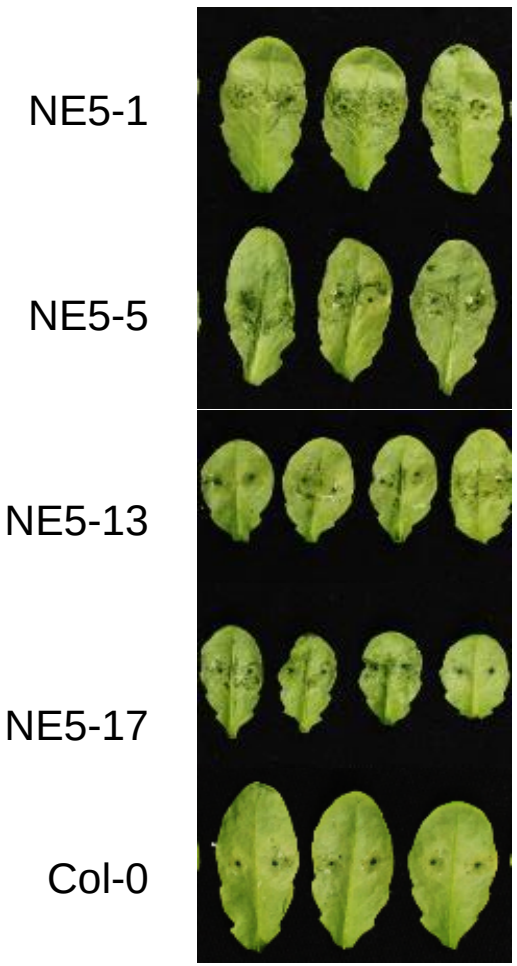
B

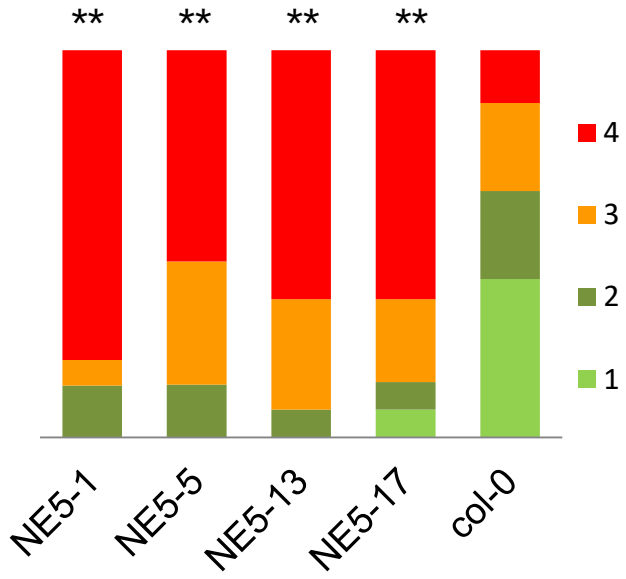
Ma*R3*
+
Avr3a

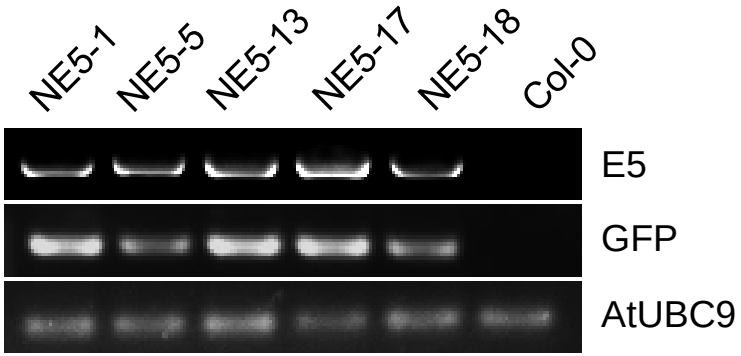


4. Use of *P. infestans* RXLR effectors

➢ Candidate core effector E5: promotes plant susceptibility



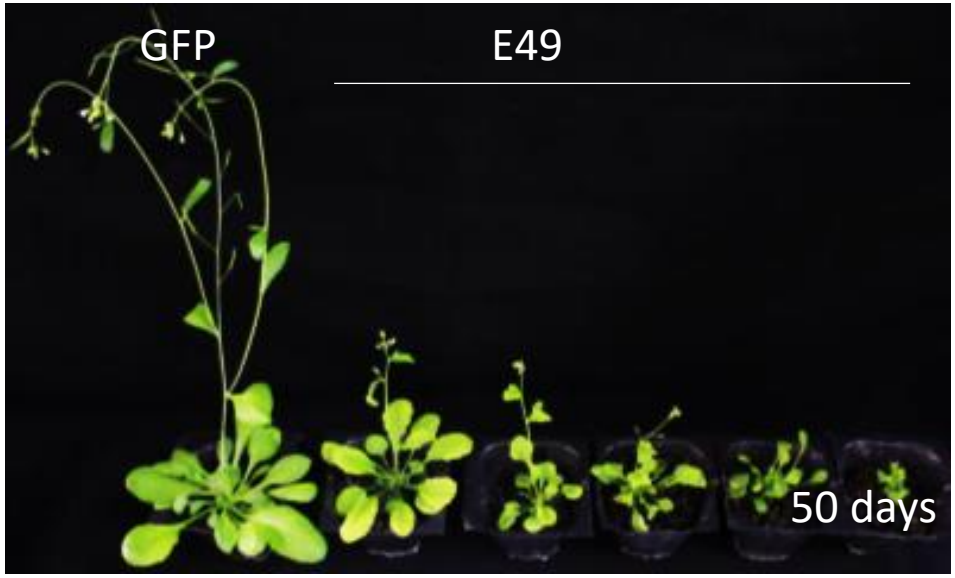


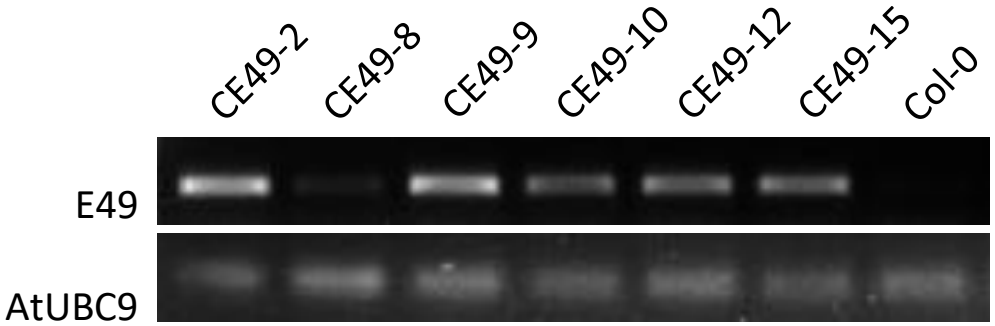


Huang et al. unpublished

4. Use of *P. infestans* RXLR effectors

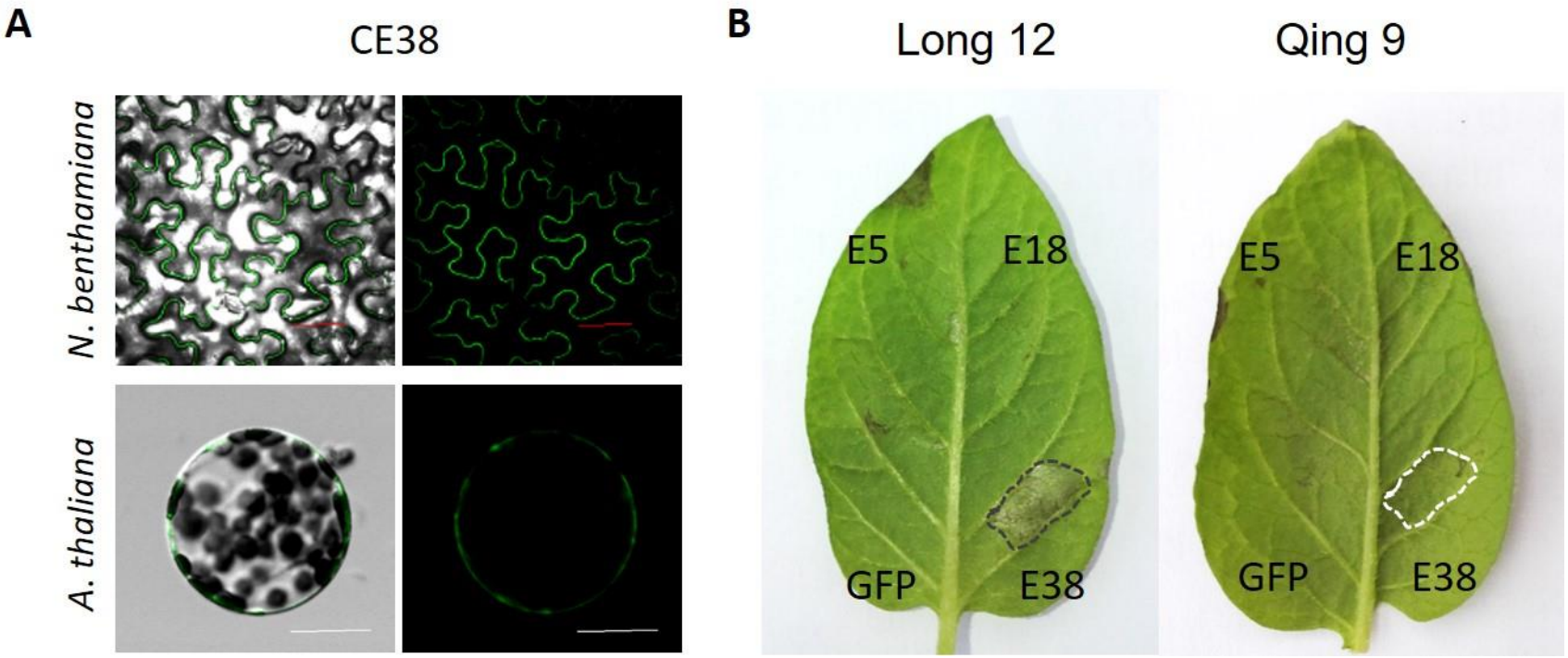
- Candidate core effector E49: suppresses plant growth





3. Use of *P. infestans* RXLR effectors

- Identification of a new *R* gene capable of recognizing candidate core effector E38

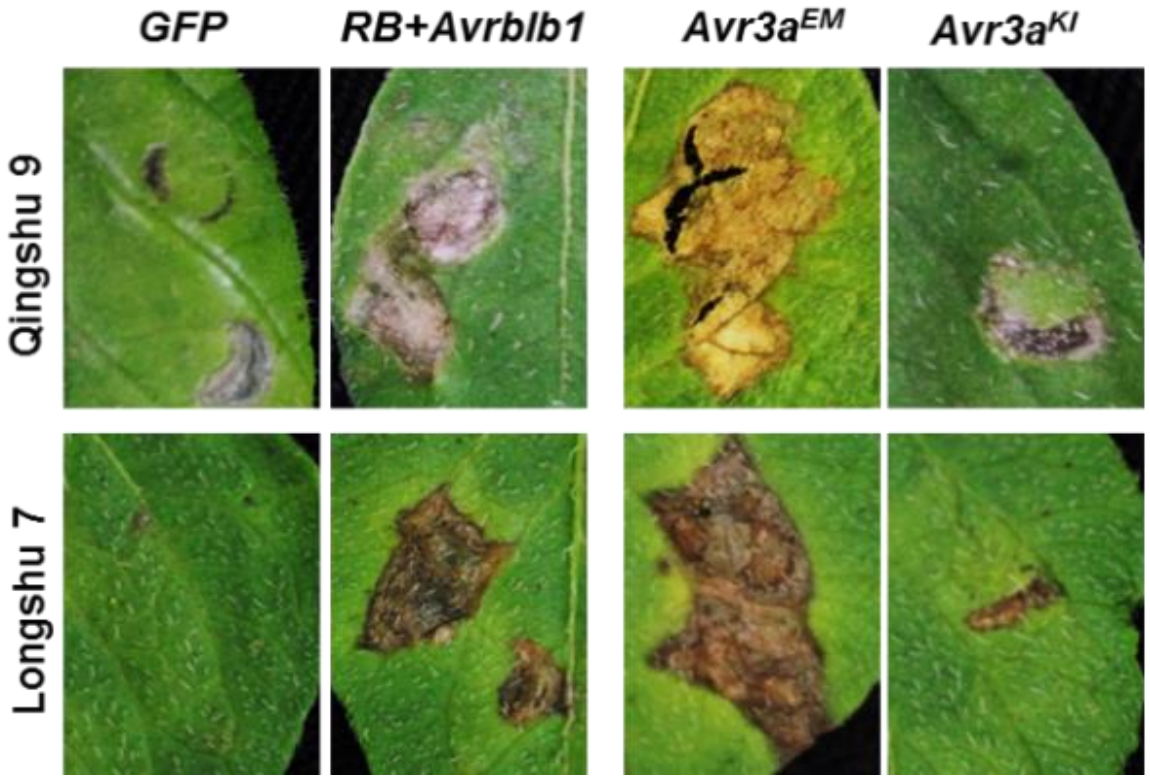


- E38 is localized on the plasma membrane
- E38 triggers potato genotype-specific cell death

Li et al. unpublished

4. Use of *P. infestans* RXLR effectors

- Qingshu9 and Longshu7 are capable of recognizing *P. infestans* core effector Avr3a^{EM}

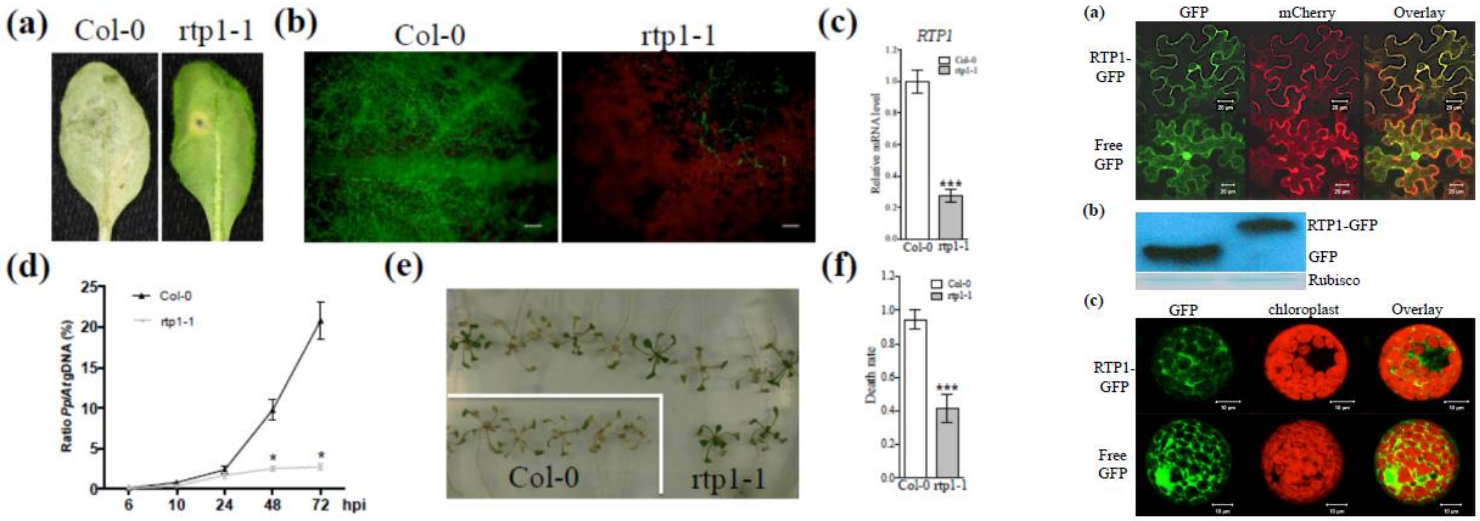


Li et al. unpublished

Genetic dissection of compatible plant-*Phytophthora* interaction

- Screening for plant proteins targeted by *Phytophthora* RXLR effectors
- Screening for plant mutants resistant to infection by *Phytophthora parasitica*

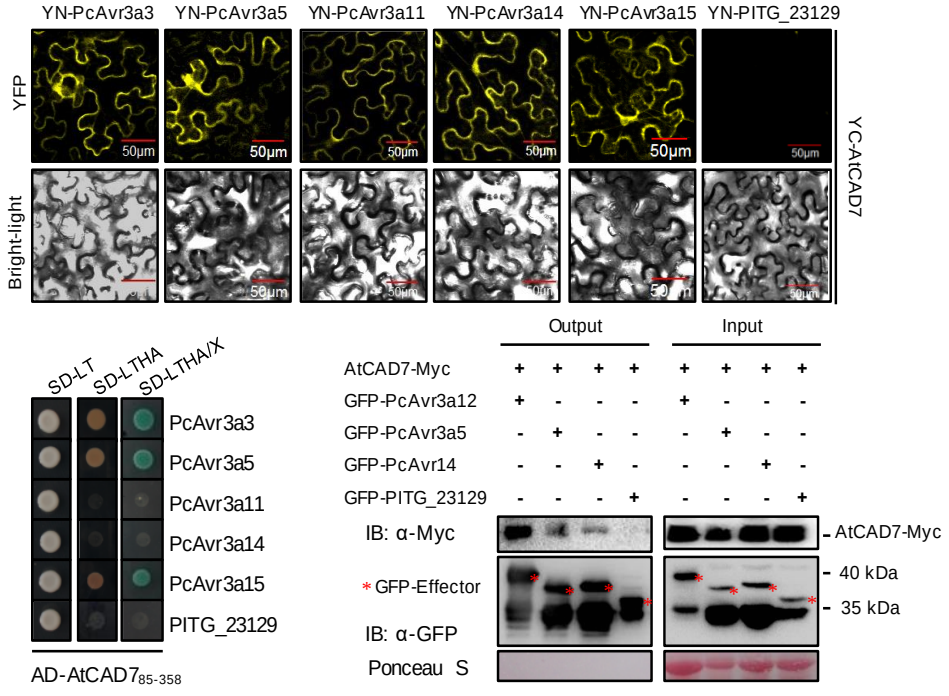
RTP1 mediates susceptibility to biotrophic pathogens



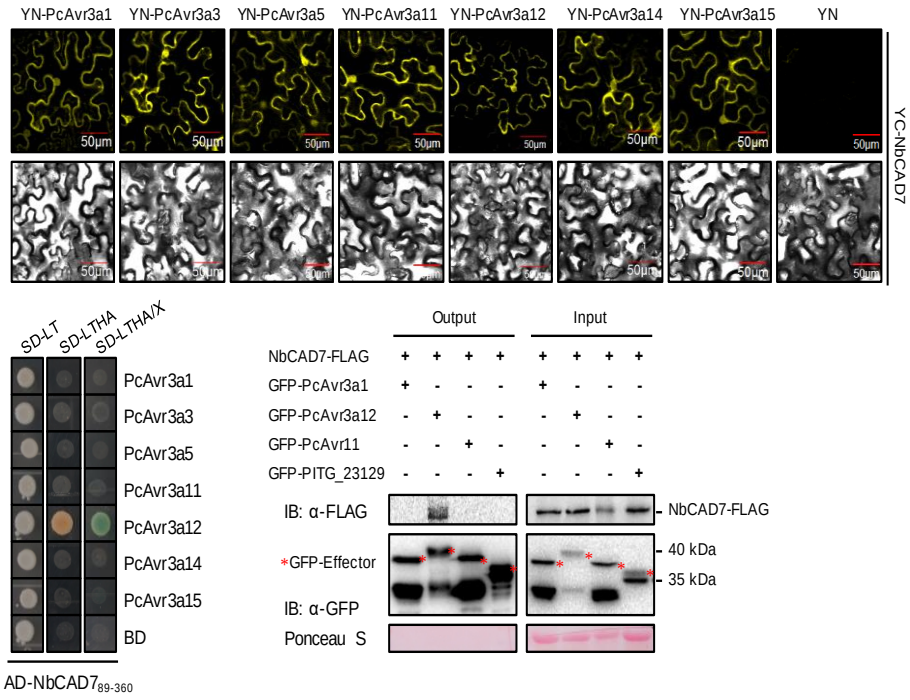
Pan et al. 2016. New Phytologist 209: 1641-1654.

5. Effector targets as novel source of disease resistance

- CAD7 (cinnamyl alcohol dehydrogenase 7): targeted by multiple *Phytophthora* Avr3a effectors



AtCAD7与辣椒疫霉菌Avr3a效应蛋白的互作

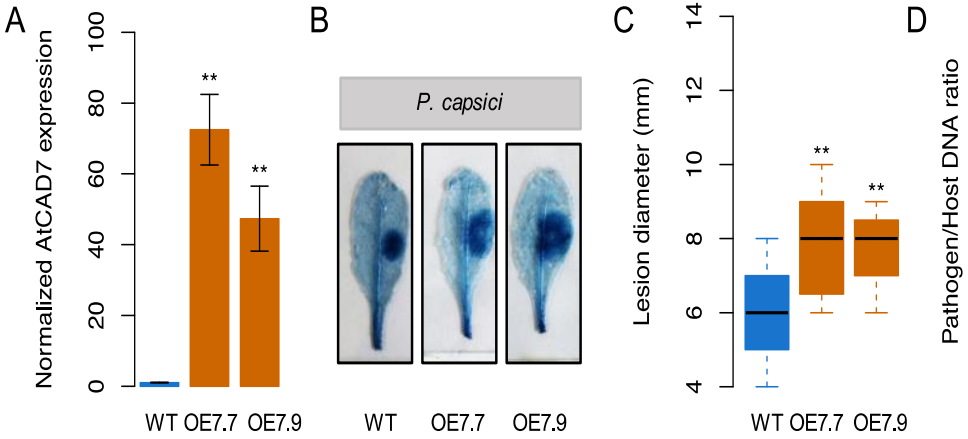


NbCAD7与辣椒疫霉菌Avr3a效应蛋白的互作

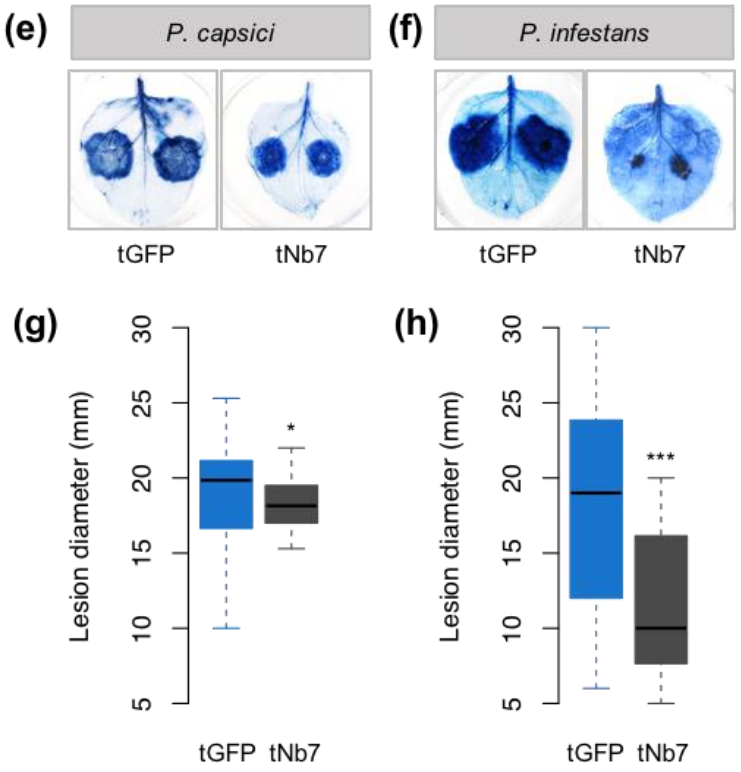
Li et al. 2019. New Phytologist

5. Effector targets as novel source of disease resistance

- *CAD7* is a negative regulator of plant immunity to *Phytophthora* pathogens



Overexpression of *AtCAD7* enhanced susceptibility of *Arabidopsis thaliana* to *Phytophthora capsici*

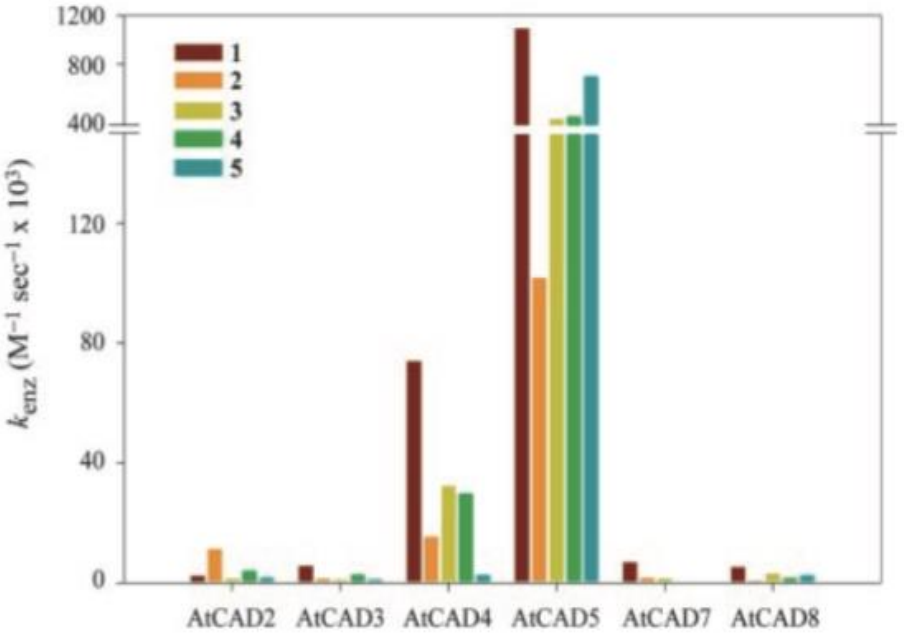


Silencing of *NbCAD7* enhanced resistance of *Nicotiana benthamiana* to *Phytophthora* pathogens

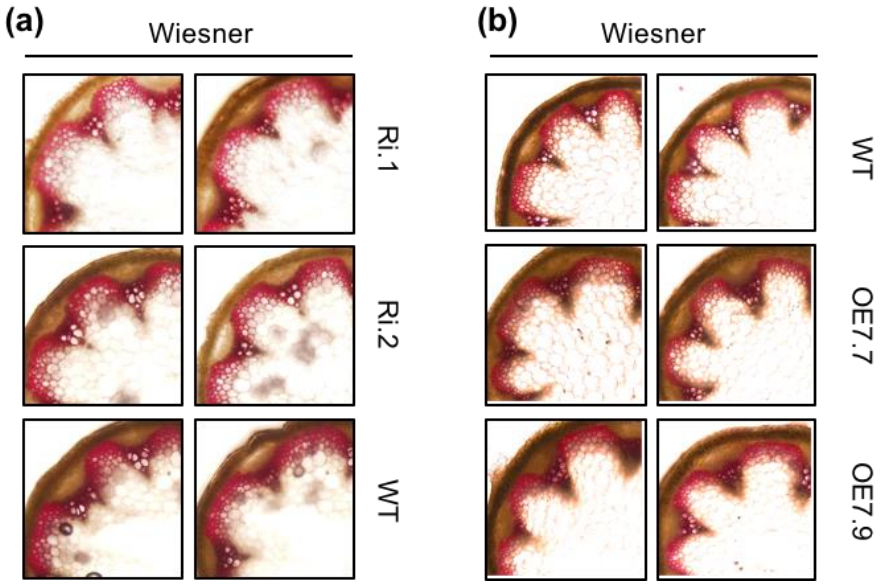
5. Effector targets as novel source of disease resistance

➢ CAD7：potential enzymatic activities

CAD4/5 CAD7/8



Analysis of CAD family members for enzymatic activities

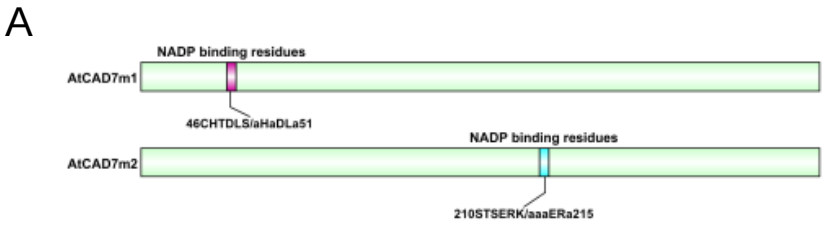


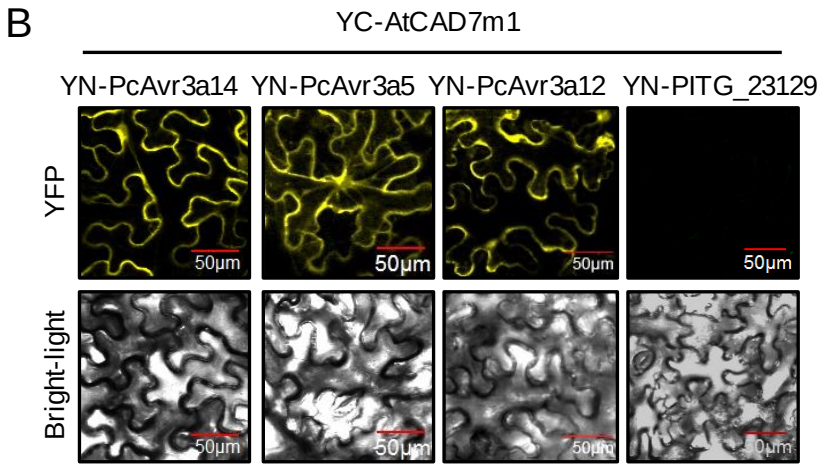
Manipulation of *AtCAD7 expression* does not affect lignin deposition

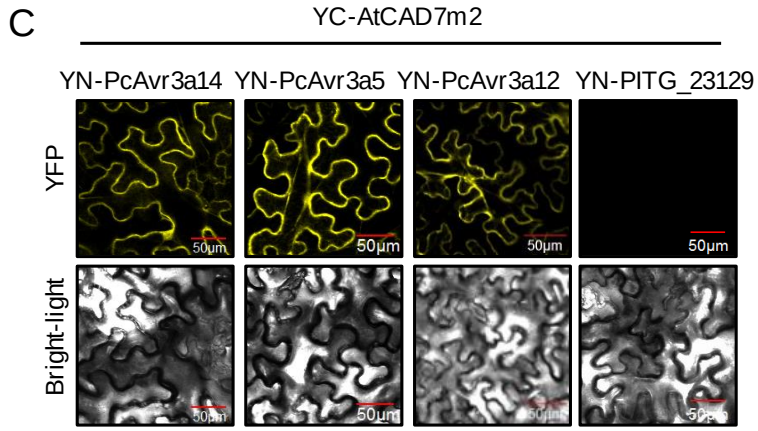
Kim et al. 2004. PNAS

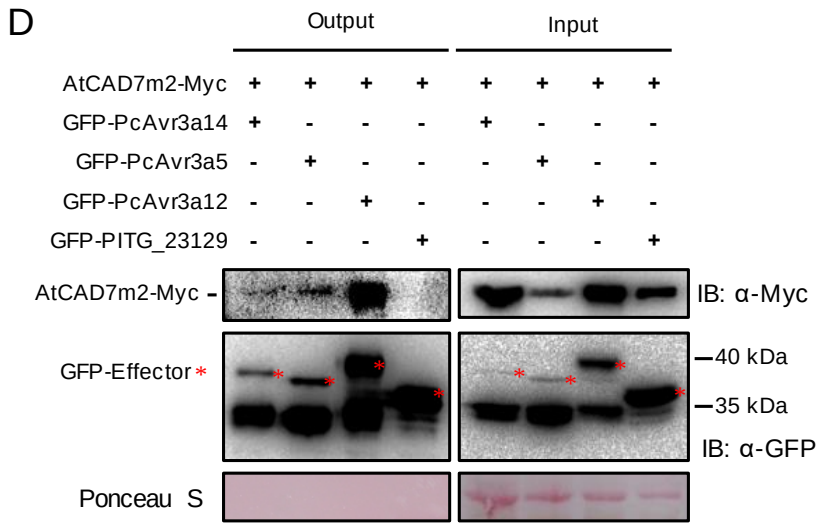
➢ Analysis of AtCAD7 proteins mutated at the predicted active enzymatic sites

Mutations in the predicted enzymatic active sites of AtCAD7 do not abolish its interactions with Avr3a effectors



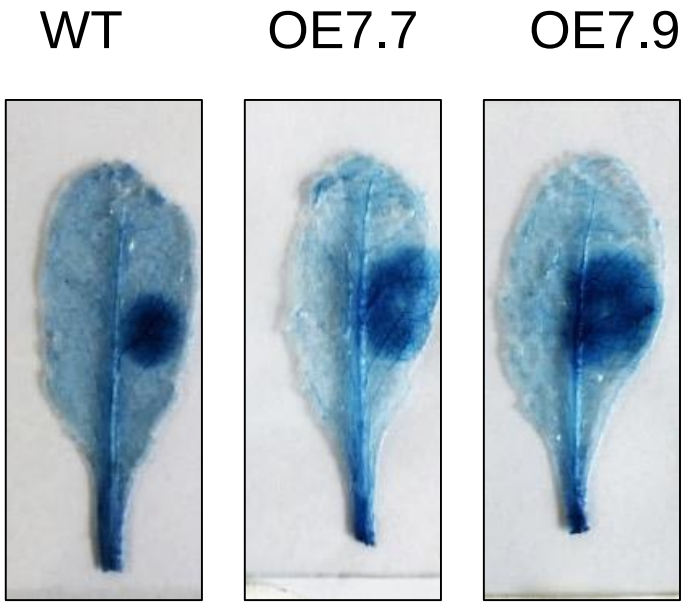


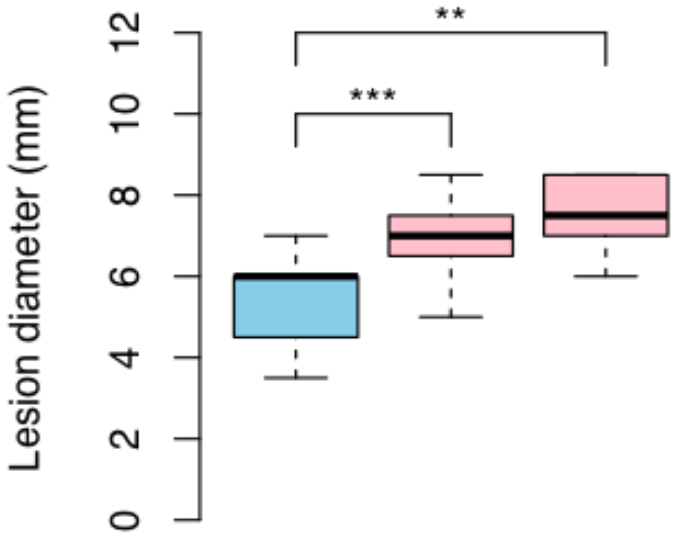


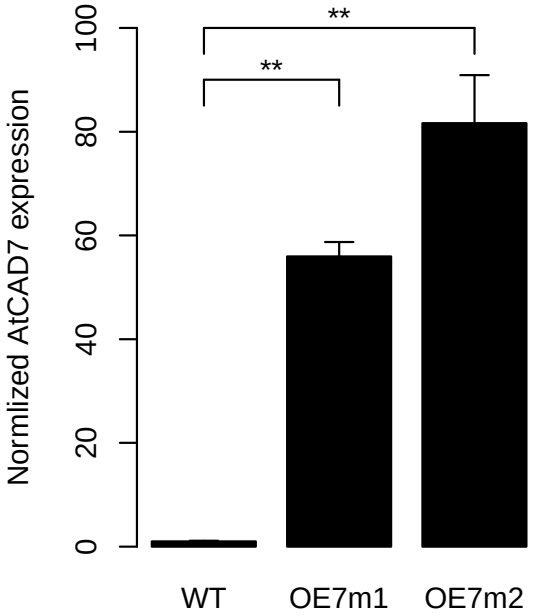


➢ Analysis of AtCAD7 proteins mutated at the predicted active enzymatic sites

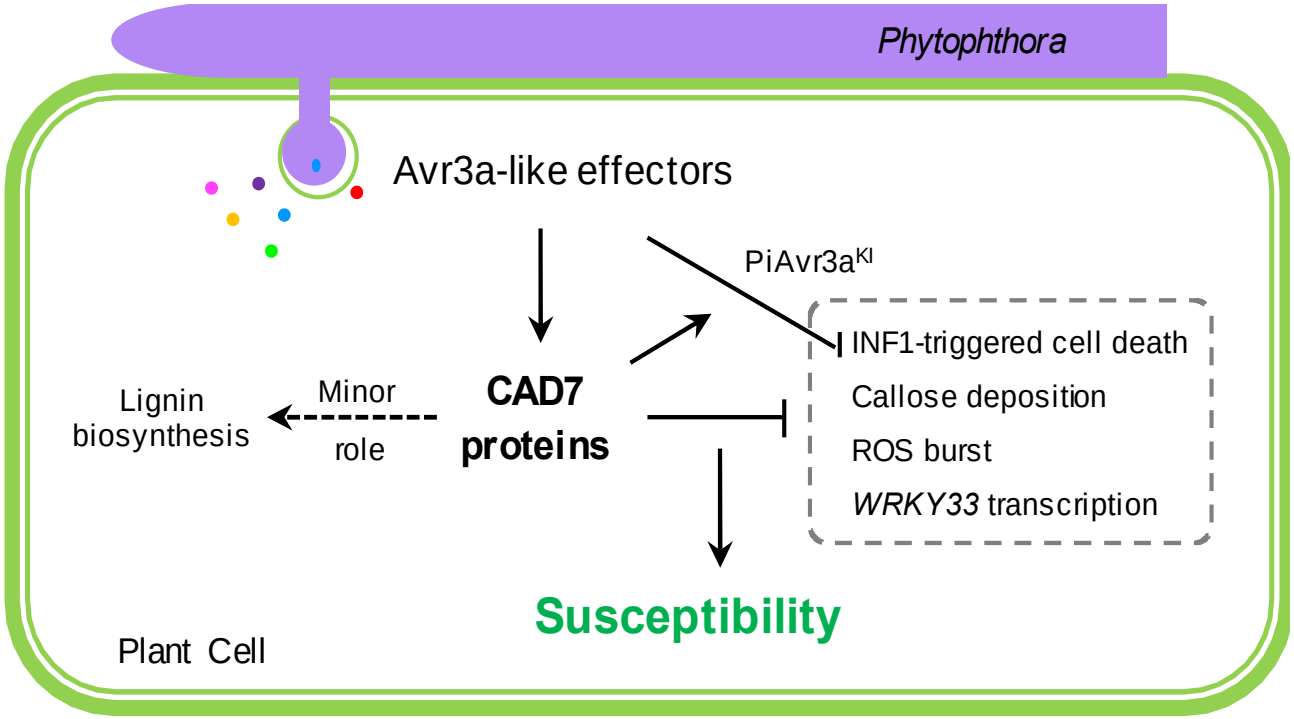
Mutations in the predicted enzymatic activity sites of AtCAD7 do not abolish its function as a negative regulator of plant immunity







- CAD7 negatively regulates plant immunity and is a common target of multiple *Phytophthora* Avr3a-like effectors

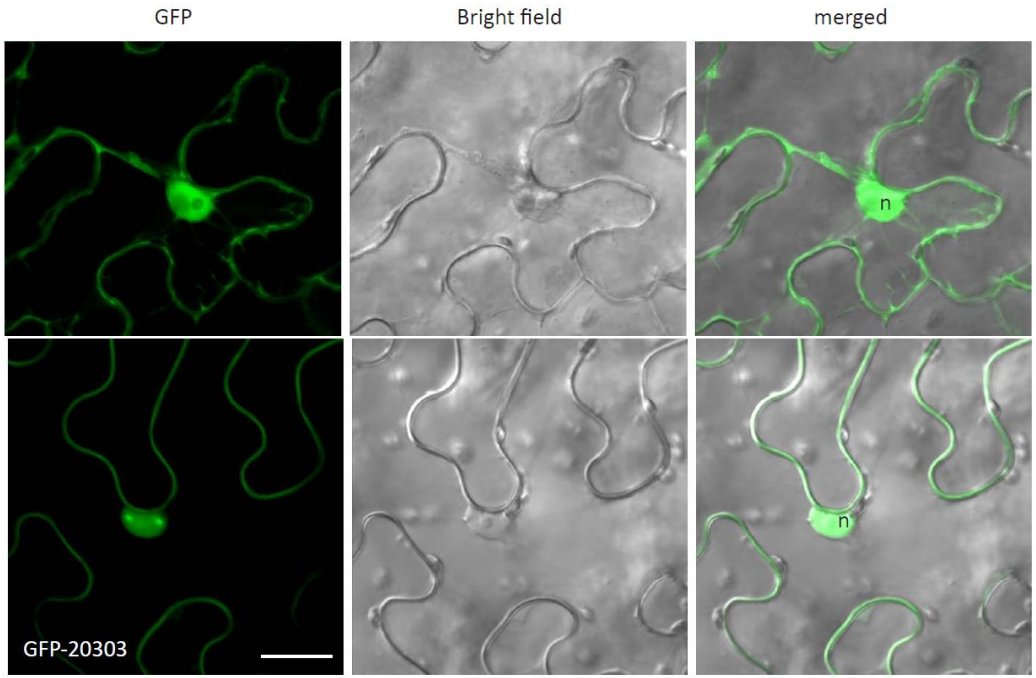


Li et al. 2019. New Phytologist

5. Effector targets as novel source of disease resistance

➢ StMKK1: targeted by *P. infestans* effector E49

- E49: a virulent variant of PiAvrblb2 with single amino acid residue change
- E49: changed subcellular localization
- E49 utilizes a different virulence mechanism from that of PiAvrblb2 by targeting and stabilizing StMKK1, a potato MAPK cascade protein.



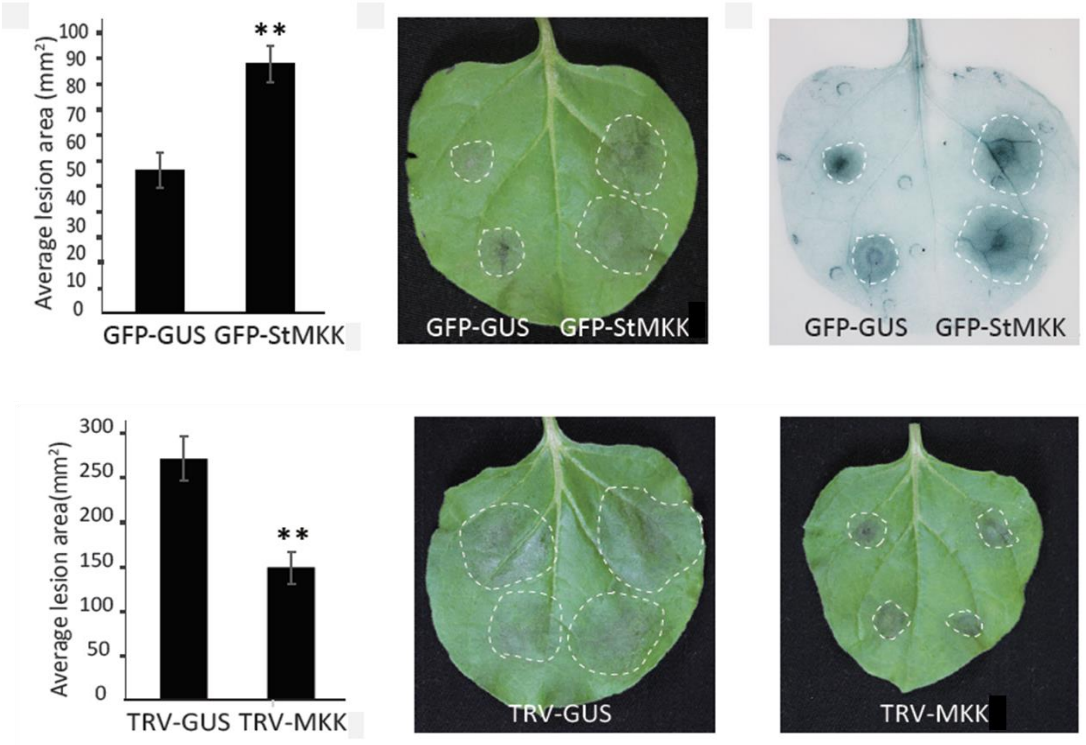
E49 localized in the nuclear and cytoplasm of the plant cell

Du et al. *unpublished*

5. Effector targets as novel source of disease resistance

- StMKK1: targeted by *P. infestans* effector E49

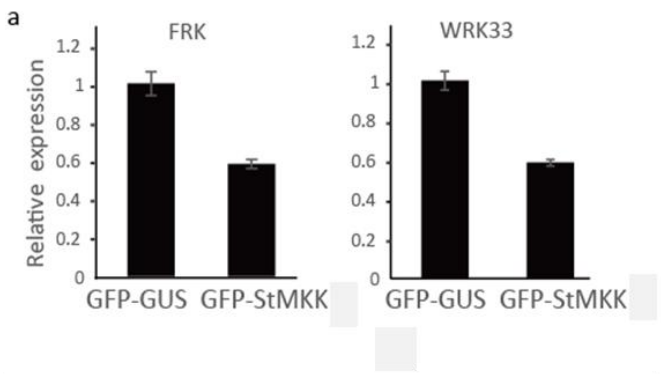
StMKK1: negatively regulates plant defence to *P. infestans*



5. Effector targets as novel source of disease resistance

- StMKK1: targeted by *P. infestans* effector E49

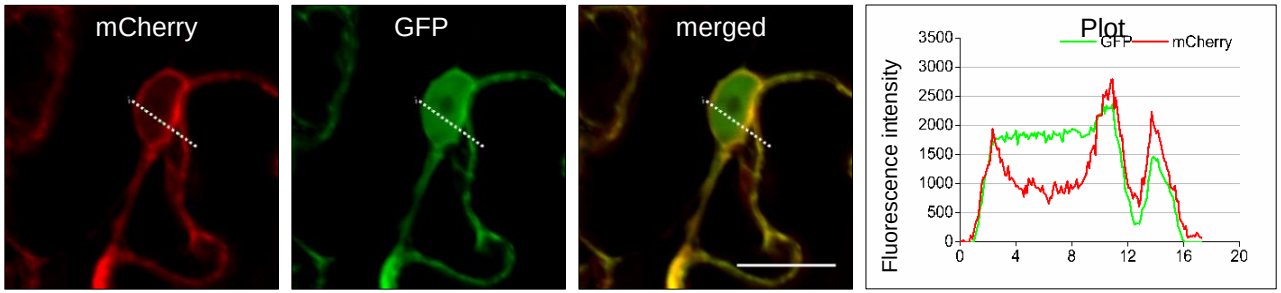
StMKK1: suppresses plant PTI responsive gene expression and ROS production



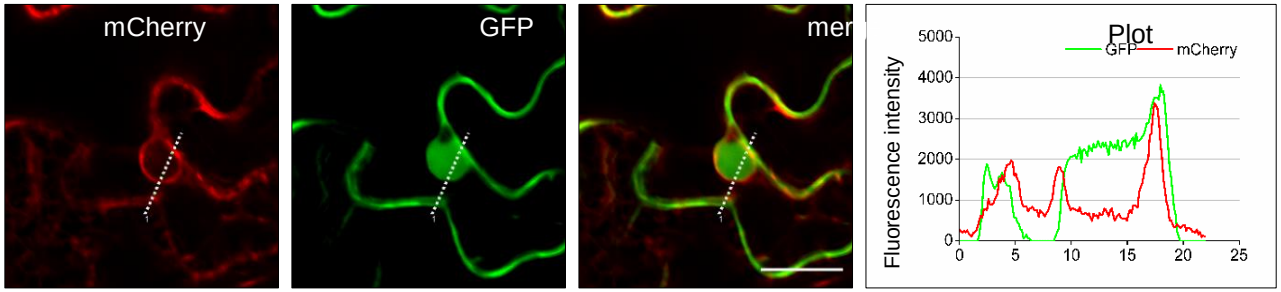
5. Effector targets as novel source of disease resistance

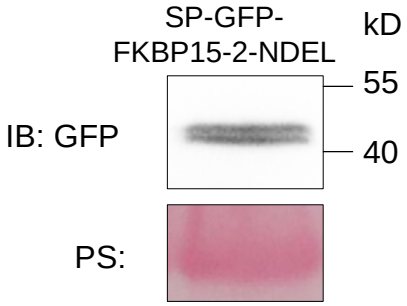
➢ FKBP15-2: targeted by effector PcAvr3a12

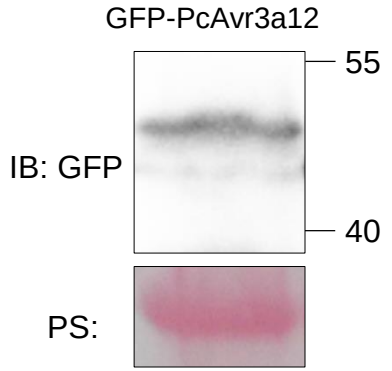
SP-mCherry-ΔFKBP15-2-NDEL + GFP-PcAvr3a12



SP-mCherry-ΔFKBP15-2-NDEL + GFP-PiAvrblb2 (control)





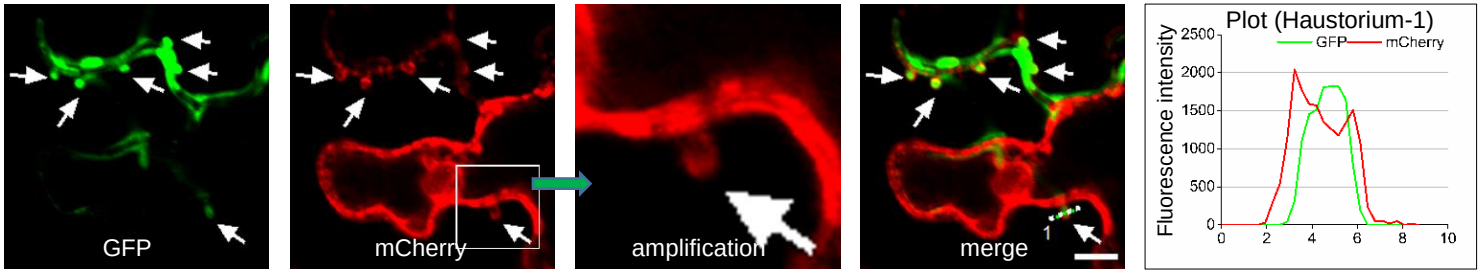


PcAvr3a12 co-localizes with FKBP15-2 on ER

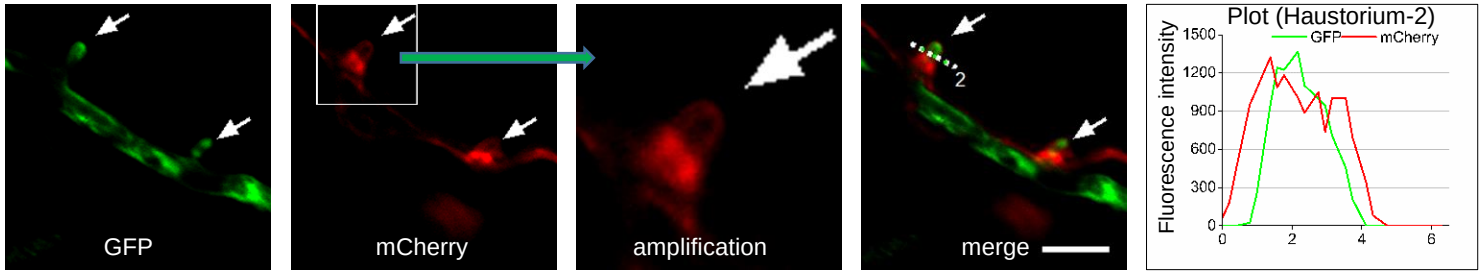
5. Effector targets as novel source of disease resistance

➢ Association of PcAvr3a12 with FKBP15-2

SP-mCherry-FKBP15-2-NDEL + GFP-labeled *P. parasitica*



mCherry-PcAvr3a12 + GFP-labeled *P. parasitica*

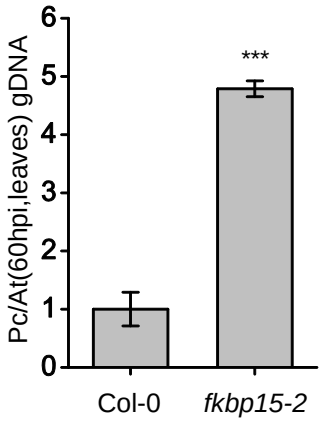


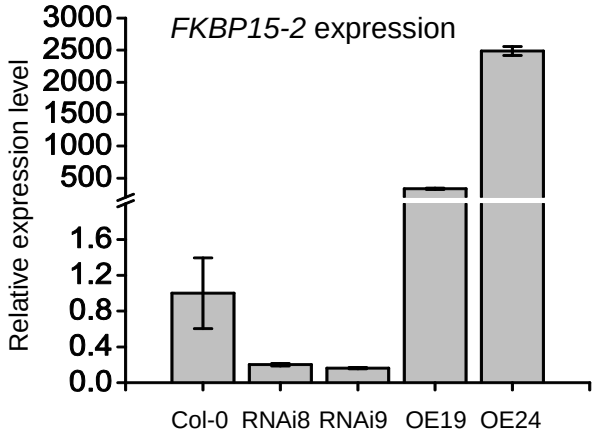
➢ PcAvr3a12 and FKBP15-2 are accumulated around haustoria

5. Effector targets as novel source of disease resistance

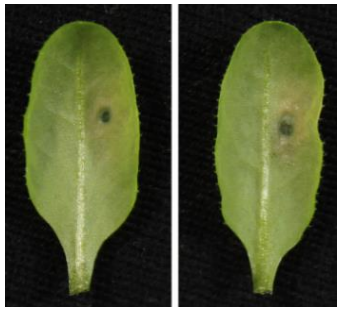
➢ *FKBP15-2* is a positive regulator of plant immunity to *Phytophthora*

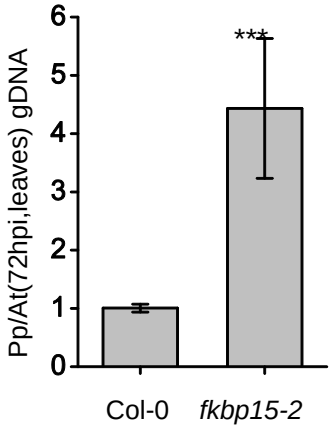
P. capsici infection



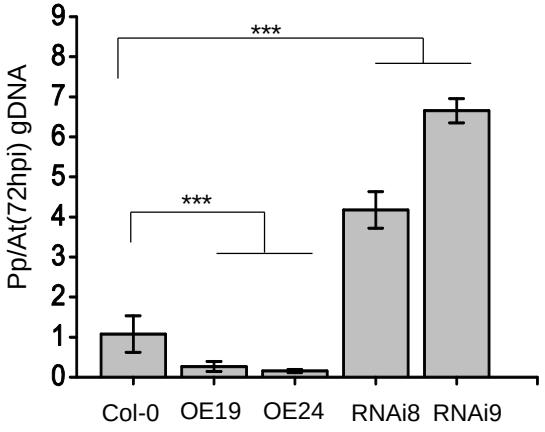


P. parasitica infection





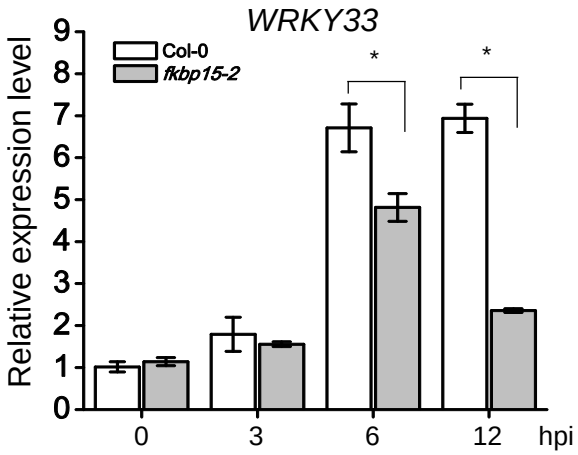
P. parasitica infection

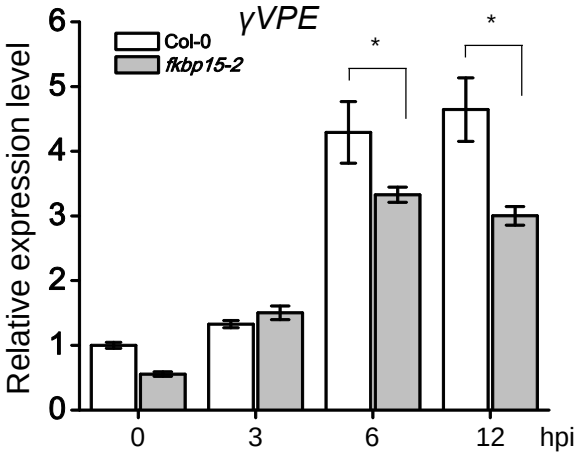


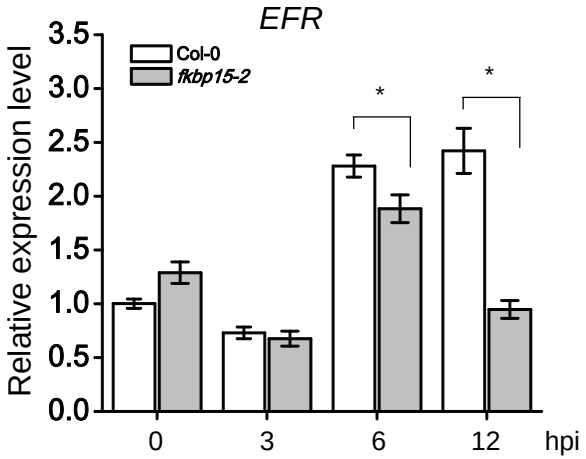
5. Effector targets as novel source of disease resistance

- *FKBP15-2* is required for ER stress sensing and in ER stress-mediated plant immunity

  - *P. capsici* infection
  - ER-cell death gene *γ-VPE*
  - UPR-mediated SAR priming marker *WRKY33* and ER-QC depended pattern-recognition receptor *EFR*

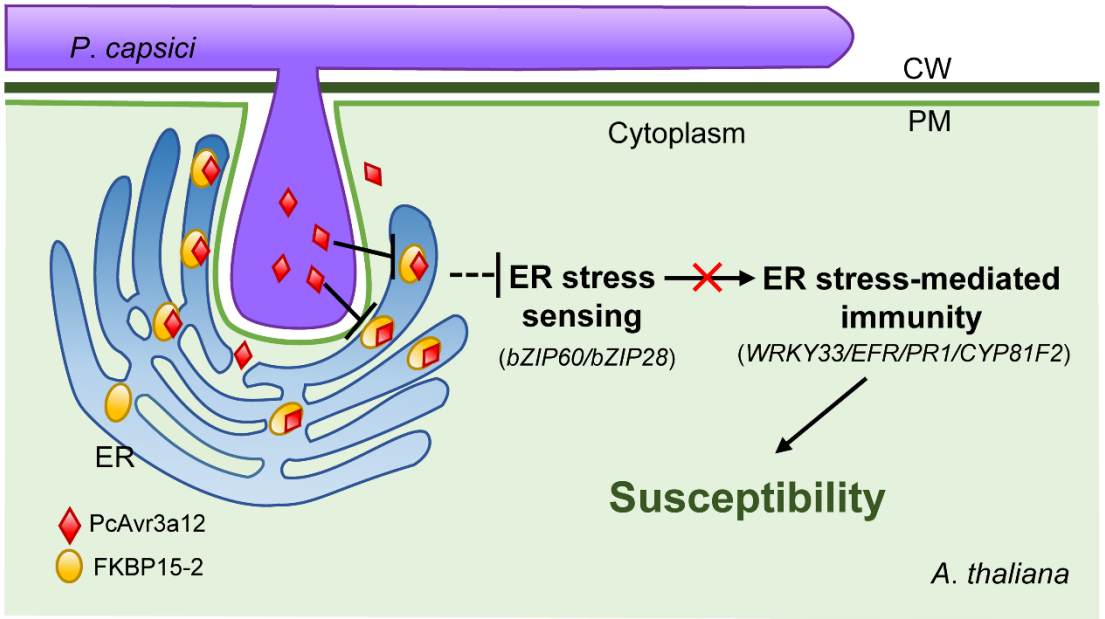






Induction of *γ-VPE*, *WRKY33*, and *EFR* was all attenuated in *fkbp15-2* mutant at early phase of *Phytophthora* infection

5. Effector targets as novel source of disease resistance



- *P. capsici* employs RXLR effector PcAvr3a12 to directly target host plant ER located PPIase FKBP15-2 to suppress plant immunity and achieve compatibility.
- FKBP15-2 is a positive regulator of plant immunity to *Phytophthora* pathogens.
- FKBP15-2 is a novel ER-located immune component and is involved in UPR and ER stress-mediated plant immunity.

Fan et al. 2018. Molecular Plant 11: 1067-1083.

6. Conclusions

- Diseases threaten sustainable crop production
- Pathogens encode numerous effectors to facilitate crop infection
- Effectors are useful for improving crop disease resistance
  - Evaluation of dominant varieties for resistance
  - Identification of new *R* genes
- Novel source of genes for improving crop disease resistance
  - Host targets of pathogen effectors
  - Genetic engineering/genome editing to disable the expression of susceptibility genes responsive to pathogen infection.

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